Biological Science

5A, 17A, NW12A, NE3A/2008G149

X-ray Crystallography of a Type III Rubisco from a Hyperthermophile

Yuichi Nishitani[1], Shosuke Yoshida[2], Masahiro Fujihashi[1], Kazuya Kitagawa[1], Takashi Doi[1], Haruyuki Atomi[2], Tadayuki Imanaka[3], Kunio Miki*[1]
[1]Department of Chemistry, Graduate School of Science, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan
[2]Department of Synthetic Chemistry and Biological Chemistry, Graduate School of Engineering, Kyoto University, Katsura, Nishikyo-ku, Kyoto 615-8510, Japan
[3]Department of Biotechnology, College of Life Sciences, Ritsumeikan University, Kusatsu, Shiga 525-8577, Japan

Introduction

The Calvin-Benson-Bassham cycle is responsible for carbon dioxide fixation in all plants, algae, and cyanobacteria. The enzyme that catalyzes the carbon dioxide-fixing reaction is ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). Rubisco from a hyperthermophilic archaeon *Thermococcus kodakarensis* (*Tk*-Rubisco) belongs to the type III group, and it shows high activity at high temperatures. We have previously determined the crystal structure of *Tk*-Rubisco in the apo-form [1]. We have also found that replacement of the entire α-helix 6 of *Tk*-Rubisco with the corresponding region of the spinach enzyme (SP6 mutant) results in an improvement of catalytic performance at mesophilic temperatures, both *in vivo* and *in vitro*, whereas the former and latter half replacements of the α-helix 6 (SP4 and SP5 mutants) do not yield such improvement [2]. We report here the crystal structures of the wild-type *Tk*-Rubisco and the mutants SP4 and SP6, and discuss the relationships between their structures and enzymatic activities.

Materials and Methods

Prior to crystallization, the purified *Tk*-Rubisco was activated by equilibrating with the buffer containing 100 mM Bicine-NaOH (pH 8.3), 10 mM $MgCl_2$, and 20 mM $NaHCO_3$ at 4°C for one day, in order to bind its reaction-intermediate analogue, 2-carboxy-D-arabinitol 1,5-bisphosphate (2-CABP). Approximately 10 mM 2-CABP was added to the activated protein solution at 4°C for one day. Crystals were obtained within a week using a reservoir solution containing 100 mM acetate buffer (pH 6.0), 80-100 mM $CaCl_2$, 5-6% (w/v) polyethylene glycol 6,000, and 10% (v/v) 2-methylpentane-2,4-diol. Diffraction data were collected using synchrotron radiation at the Photon Factory (BL5A, BL17A, AR-NW12, and AR-NE3A). The structures were determined by the molecular replacement method using the apo-enzyme structure (PDB ID: 1GEH) as a search model.

Results and Discussion

We have determined the crystal structures of the wild-type *Tk*-Rubisco, and the mutants SP4 and SP6 in complex with 2-CABP at 2.30 Å, 2.09 Å, and 2.36 Å, respectively [2]. The resolutions are improved compared to that of the wild-type apo enzyme (2.8 Å). The active-site loop 6, which has been disordered in the apo form, can be observed in the electron density map.

A comparison among these structures shows the movement and the increase of temperature factors of α-helix 6 induced by four essential factors (Figure 1). We thus supposed that an increase in the flexibility of the α-helix 6 and its neighbor loop 6 regions was important to increase the catalytic activity of *Tk*-Rubisco at ambient temperatures. Based on this structural information, we constructed a new mutant, SP5-V330T, which was designed to have significantly greater flexibility in the above region, and it proved to exhibit the highest activity among all mutants examined to date. The thermostability of the SP5-V330T mutant was lower than that of wild-type *Tk*-Rubisco, providing further support on the relationship between flexibility and activity at ambient temperatures.

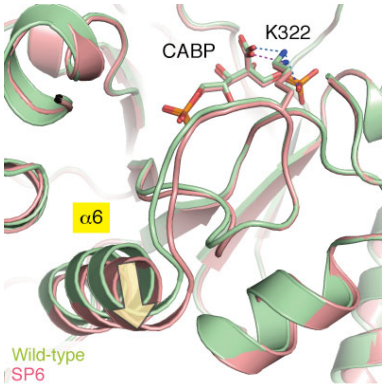

Figure 1. Movement of α-helix 6.

References

[1] K. Kitano, N. Maeda, T. Fukui, H. Atomi, T. Imanaka, K. Miki, *Structure* **9**, 473 (2001).
[2] S. Yoshida, H. Atomi, T. Imanaka, *Appl. Environ. Microbiol.* **73**, 6254 (2007).
[3] Y. Nishitani, S. Yoshida, M. Fujihashi, K. Kitagawa, T. Doi, H. Atomi, T. Imanaka, K. Miki, *J. Biol. Chem.* **285**, 39339 (2010).

* miki@kuchem.kyoto-u.ac.jp